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PRINCIPAL INVESTIGATOR: Paula T. Hammond

CONTRACTING ORGANIZATION: Koch Institute of Integrative Cancer Research, Massachusetts Institute of Technology, CAMBRIDGE, MA 02139

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email: hammond@mit.edu

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14. ABSTRACT

We propose new modular polymer systems that can generate targeted siRNA in a new stable and highly potent form using safe polymeric systems in place of viruses, which are known to yield undesirable side-effects. Key to these nanomaterial systems is the ability to "program-in" to a single delivery system specific combinations of chemotherapy drugs with siRNA and molecular inhibitors shown to be effective in ovarian cancer, thus providing the opportunity for highly synergistic couplings of therapeutics delivered in a safe and effective manner. This goal will be accomplished using the biologically intuitive design of nanoparticles using three key innovations, which are examined in Specific Aims 1 through 3: concatenated siRNA systems (csiRNA), layer-by-layer nanoparticle delivery of combination therapies, and polypeptide based linear-dendritic copolymers for siRNA encapsulation. New developments with csiRNA and continued work with the related RNAi microsponges is promising, and greater understanding of these systems will enable the investigation of targets identified by Michael Birrer, including FGF18, that will synergize with the innate immune response of the tumor cells to achieve a highly effective treatment. We continue to develop LbL nanoparticle systems designed specifically to deliver a chemotherapy drug for DNA damage from its interior, and siRNA that can block genes that promote or enable cell survival in the presence of the drug from its exterior. New work in this area includes the successful knockdown of EZH2 and other select targets as a result of collaborations with the Drapkin lab. In vitro work suggests potential for efficacy in these systems, and we will examine the efficacy in vivo in the upcoming year, in particular when using LbL stealth targeting outer layers also developed during this grant period. We have developed a cluster-ligand system that seems to lead to downregulation of pathways associated with tumor cell growth and proliferation, which is key to the objective of Aim 3. Our synthetic polypeptide linear and linear-dendritic block copolymer have been demonstrated in vivo to yield promising results in a high grade serous ovarian cancer model, and these systems will be further examined to confirm proposed mechanisms of action.

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Nano-siRNA Particles and Combination Therapies for Ovarian Tumor Targeting
OCRP Teal Innovator Statement of Work

PI: Paula T. Hammond, Department of Chemical Engineering
Koch Institute of Integrative Cancer Research
Massachusetts Institute of Technology

INTRODUCTION:

One of the key issues in epithelial ovarian cancer is the low clinical response to chemotherapy, and the high drug resistance found in the majority of patients who experience relapse[1,2]. These issues can be addressed with intelligent approaches to combination therapies that address the molecular pathways involved in drug resistance using gene regulation; siRNA is a particularly promising approach that has not yet reached clinical translation due to issues of delivery. Challenges include the ability to effectively condense, encapsulate and protect small charged nucleic acids, and the need to gain highly selective molecular targeting[3]. We propose new modular polymer systems that can generate targeted siRNA in a new stable and highly potent form using safe polymeric systems in place of viruses, which are known to yield undesirable side-effects. Key to these nanomaterial systems is the ability to "program-in" to a single delivery system specific combinations of chemotherapy drugs with siRNA and molecular inhibitors shown to be effective in ovarian cancer, thus providing the opportunity for highly synergistic couplings of therapeutics delivered in a safe and effective manner. This goal will be accomplished using the biologically intuitive design of nanoparticles using three key innovations, which are examined in Specific Aims 1 through 3:

1) The use of a new and highly effective means of RNAi polymerization and self-assembly to load high quantities of microRNA or siRNA sequences specifically targeting pathways to cell drug resistance[4-6] into condensed nanoparticles in a form that protects the siRNA from degradation, and releases it to the cell in high yield;

2) The design of "sheddable layers" on nanoparticle surfaces for synergistic combination drug therapy using modular chemical linkages to enable the tumor microenvironment pH or other tumor cell-triggered sequential release of siRNA, followed by inhibitors and chemotherapy drugs from the nanoparticles;

3) The presentation of ligand on nanoparticle surfaces in cluster arrangements that enable optimization of ligand presentation at low overall ligand levels and maximized tumor cell selectivity.

These concepts can be integrated to generate highly functional LbL nanoparticle systems specifically tuned to release drug in the ovarian tumor microenvironment, and designed with therapeutic combinations selected to address the challenges of ovarian cancer inhibition and drug resistance.

KEYWORDS: siRNA delivery, targeted nanoparticle, combination therapy, self-assembly, cancer nanotechnology,

OVERALL PROJECT SUMMARY:

Specific Aim 1: Self-packaging siRNA

Aim 1 Current Objectives: To address the barriers of RNA delivery, we have reported for the first time the polymerization of RNAi sequences in a form that folds into its own pre-packaged structure for delivery[7]. We use a means of synthesizing polymeric siRNA termed rolling circle transcription (RCT) with a polymerase to yield large macromolecular species that consist of

RNAi targeting sequences separated by RNA sequences that are rapidly cleaved by Dicer molecules in the cytosol (see Figure 1a). These RNA structures consist of long, repeating siRNA sequences, termed concatemeric siRNA (csiRNA), contained within self-assembled sponge-like particles templated from inorganic nanocrystals, or isolated in polymeric form. By combining these materials with different polymers we obtain stable nanoparticles that show prolonged blood circulation, accumulate in tumors, and can silence gene expression in cancer cells. The sponge-like microporous particles we refer to as RNAi microsponges (RNAi MS) each contain many thousands of copies of siRNA in a single unit (see Figure 1b). When a layer of a polycation is adsorbed onto the RNAi microsponges, they are compacted to positively charged nanoparticles that can deliver up to orders of magnitude higher amounts of siRNA than a traditional commercial lipid-based cationic vehicle (Figure 1c). The RNAi can also be removed from the microsponge template and isolated; these large chain concatenated siRNA (csiRNA) can be assembled with block copolymers or other polymeric complexes to also derive unique RNAi carrier systems in which the siRNA is preserved in a more stable form, and may exhibit modified biodistribution that can be favorable to tumor accumulation and sustained transfection within tumors. We hypothesize that each of these resulting self-assembled systems enable the delivery of a much higher amount of siRNA using a significantly lowered dose, thus improving siRNA knock-down efficacy while greatly increasing safety.

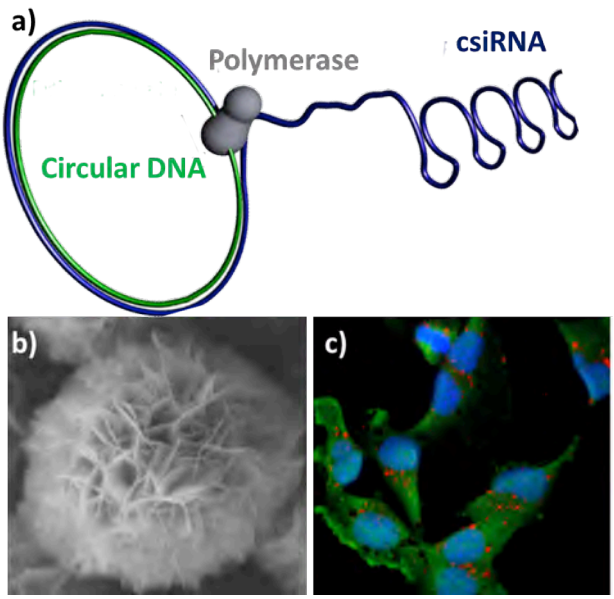

Figure 1. Novel approaches for delivering siRNA to ovarian cancer cells. (a) Cartoon representing the rolling circle transcription method for making concatemeric siRNA (csiRNA). (b) Scanning electron micrograph of an RNA microsponge particle formed during the rolling circle transcription reaction (particle is ~ 2 μm). (c) Confocal fluorescence microscope image showing ovarian cancer cells that have taken up RNA-containing nanoparticles (red).

The use of key discovered genetic targets for ovarian cancer (OC) will be coupled with this powerful new delivery platform through collaborations with Dr. Michael Birrer, M.D., PhD, who has participated in large scale studies of genome expression in ovarian cancer that have identified key genetic subgroups associated with high grade serous tumors[8], including those characteristic of drug resistant phenotypes of recurrent ovarian cancer. Two or more specific siRNA or micro-RNA sequences that silence or regulate these genes will be selected and incorporated into the RNAi microsponge (RNAi MS) to address resistant tumors. Because the sponge platform can be designed to incorporate 2, 3 or more siRNA sequences, unique combinations of siRNA will also be explored. Our efforts investigate the use of these nanoparticles to target genes that have been implicated in the progression of ovarian cancer, including MAGP2 and FGF18. We have selected FGF18 in place of previously considered IKKβ, as the FGF18 gene is now viewed by our collaborator, Dr. Michael Birrer, as the more promising target for oncological effect and possible synergy with MAGP2; however, in future work we may also explore these or other pathways that may prove effective oncological targets based on our earlier results and any new information about ovarian cancer genetic pathways.

Aim 1 Results, Progress, and Accomplishments with Discussion:

1.1 Innate cytotoxicity of csiRNA in ovarian cancer cells

While testing the knockdown efficacy of csiRNA formulations we noted striking cytotoxic effects that were independent of gene knockdown: this led us to screen the cytotoxicity of csiRNA formulations in a panel of ovarian cancer cell lines. csiRNA caused a dose-dependent decrease of cell viability in all of the cell lines that were tested: namely SKOV3, CAOV3, OVCAR4, JHOS4, and UCI101 cells (Figure 2a). We compared the cytotoxic effects of csiRNA with monomeric siRNA (21 bp) and poly-I:C, which is a large dsRNA analogue used primarily as an immunostimulatory agent. The cytotoxicity of csiRNA was found to be an order of magnitude higher than 21 bp siRNA with the same double-stranded sequence, suggesting that the large size of the csiRNA molecules contributes to their cytotoxic effects. Overall, the greatest toxicity was observed when csiRNA was complexed to lipids, however, considerable toxicity was also observed for csiRNA complexed to cationic polymers (PEI and Mirus cationic polymer transfection reagents).

The cytotoxic effects of poly-I:C have been previously reported and studied for ovarian

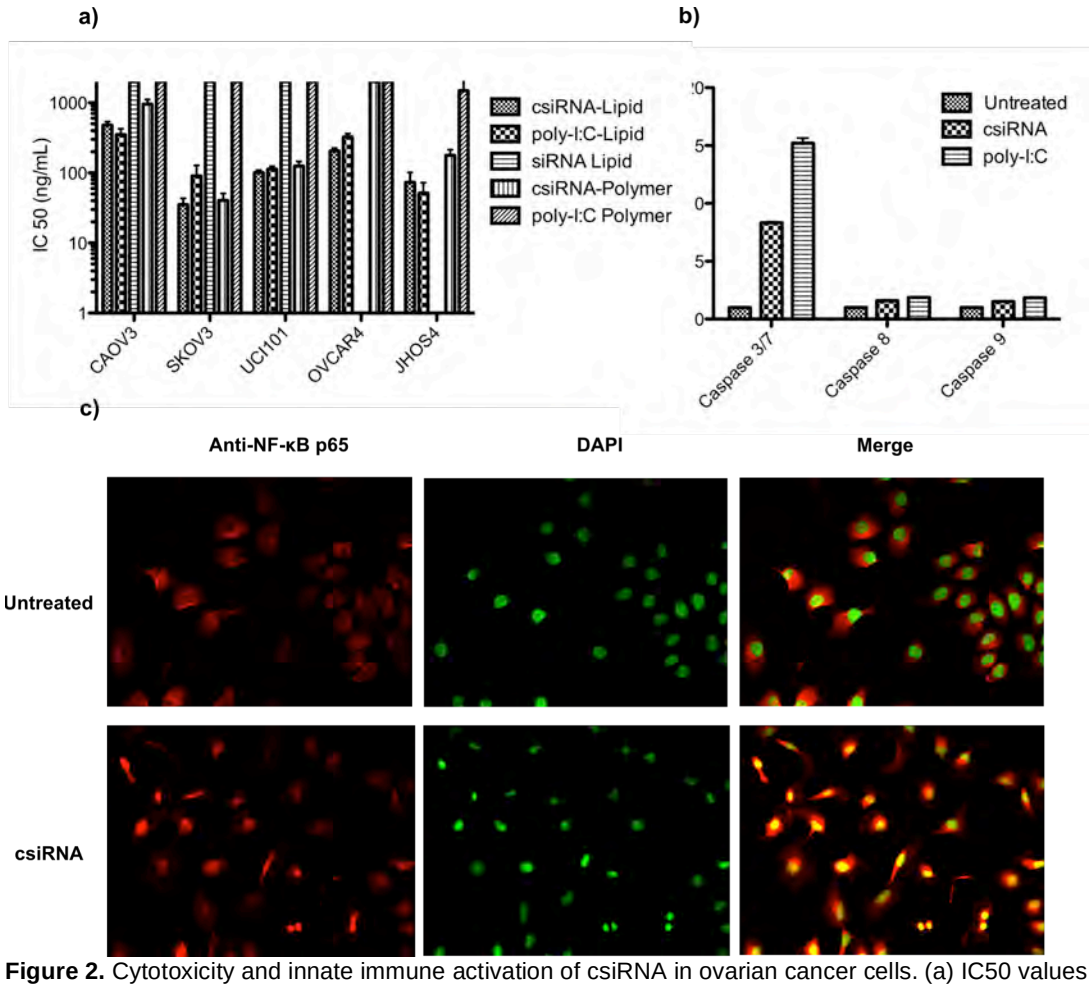

Figure 2. Cytotoxicity and innate immune activation of csiRNA in ovarian cancer cells. (a) IC50 values for csiRNA and other RNA formulations in five ovarian cancer cell lines (lipid = Lipofectamine 2000; polymer = Mirus Trans-ITX2). (b) Caspase activity measured in SKOV3 cells 16 h after treatment with 300 ng/mL csiRNA or poly-I:C lipid complexes. (c) Fluorescence microscopy of SKOV3 before and after (4 h) treatment with csiRNA-lipid complexes. Images show the nuclear translocation of NF-κB following treatment.

cancer along with a variety of other cancer types. The cytotoxicity of csiRNA and poly-I:C were virtually identical when complexed with cationic lipids (geom. mean IC_{50} across all cell lines = 156 and 145 ng/mL, respectively). In contrast, when complexed with the Mirus cationic polymer csiRNA was found to be much more toxic than poly-I:C (geom. mean IC_{50} = 379 and 4977 ng/mL, respectively). Poly-I:C is thought to induce apoptosis in cancer cells by activating innate immune receptors, which can lead to caspase-mediated cell death. We measured Caspase 3/7 activation in SKOV3 cells following treatment with 300 ng/mL csiRNA and found a 9-fold increase in activity after 16 h, consistent with the induction of apoptosis (Figure 2b). In addition to the effector Caspase 3/7, we also observed activation of the initiator caspases 8 and 9, both of which have been implicated in initiating poly-I:C induced apoptosis in cancer cells.

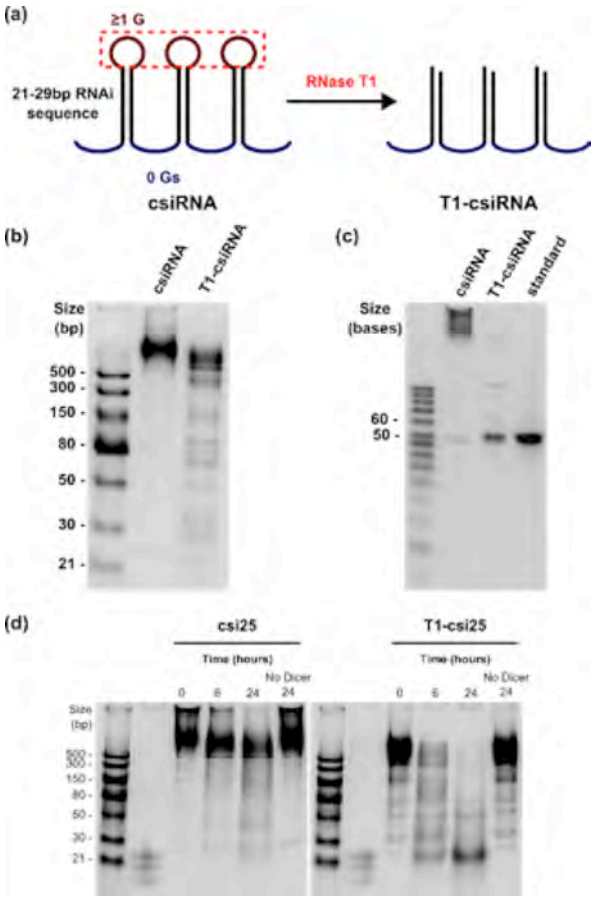

Figure 3. (a) Schematic of open-ended csiRNA produced by RNase T1 cleavage of csiRNA. (b) Analysis of csiRNA before and after RNase T1 cleavage by native 15% PAGE. (c) Analysis of T1-csiRNA and standard RNA with the predicted sequence by denaturing 15% PAGE. (d) Dicer cleavage assay of csiRNA and T1-csiRNA with 25 bp stem (csi25 and T1-csi25, respectively), analyzed by 15% native PAGE.

One of the phenotypic hallmarks of innate immune activation is NF-κB nuclear translocation. To see if csiRNA is capable of activating the NF-κB pathway, we treated SKOV3 cells for 4 h with csiRNA-lipid complexes and stained the cells with an NF-κB antibody (Figure 2c). Comparing the cells before and after treatment, we observed a clear increase in nuclear NF-κB, as shown by the overlap of NF-κB-antibody (red) and nuclear stain (green) fluorescence. This was quantified by taking the ratio of median nuclear NF-κB-associated fluorescence over cytoplasmic NF-κB-associated fluorescence, which increased by a factor of 2.5 relative to the untreated cells; this increase was even greater than that caused by poly-I:C (~2-fold increase).

We have demonstrated that csiRNA is capable of activating innate immune pathways and inducing apoptosis in ovarian cancer cells with, depending on the mode of delivery, either similar or much higher potency compared to poly-I:C. Several receptors could be involved in mediating these effects including RIG-1, MDA5 and/or TLR3. Activation of these receptors is known to depend on various structural features of RNA including double-stranded regions and overall length. Unlike poly-I:C, the precise sequence and structure of csiRNA can be readily programmed, which could be used to fine-tune its cytotoxic activity; furthermore, csiRNA can be designed for RNAi. **Combining the innate cytotoxicity of csiRNA with judiciously chosen RNAi targets is expected to lead to more potent effects: these investigations will be undertaken in year 3.**

1.2 Improving the knockdown efficiency of csiRNA

Parallel to our investigations on the innate toxicity of csiRNA, we carried out experiments to optimize its gene knockdown efficiency. Although unmodified csiRNA is able to silence gene expression in ovarian cancer cells to a certain extent, the required doses are typically within the

cytotoxic range thus making it difficult to observe phenotypic changes specifically related to gene silencing.

As Dicer recognizes open ends of double stranded RNA for processing, the lack of open ends in csiRNA may contribute to inefficient intracellular processing and thus limited gene silencing. To improve Dicer cleavage efficiency, we designed an open-ended csiRNA via cleavage of one of the loops in specifically designed csiRNA structures by RNase T1, which preferentially cleaves single stranded RNA after guanosine residues. Through a series of experiments we discovered that csiRNA co-transcriptionally folds into a periodic hairpin structure as illustrated in Figure 3a. Given this structure, we designed csiRNA molecules with one loop in each repeat containing Gs and the other loop containing no Gs. By controlling the loop sizes and sequences, we could successfully manipulate the csiRNA to fold with the G-containing loops in the "top" hairpin positions for cleavage by RNase T1: this configuration is such that treatment with RNase T1 yields an open-ended csiRNA that is predicted to retain its concatermeric form through base-pairing interactions (Figure 2a). We confirmed that the concatemeric form was retained after RNase T1 cleavage by native polyacrylamide gel electrophoresis (PAGE), and that the constituent fragments matched a standard hairpin RNA with the same predicted size and sequence when run on a denaturing PAGE gel (Figure 3b-c).

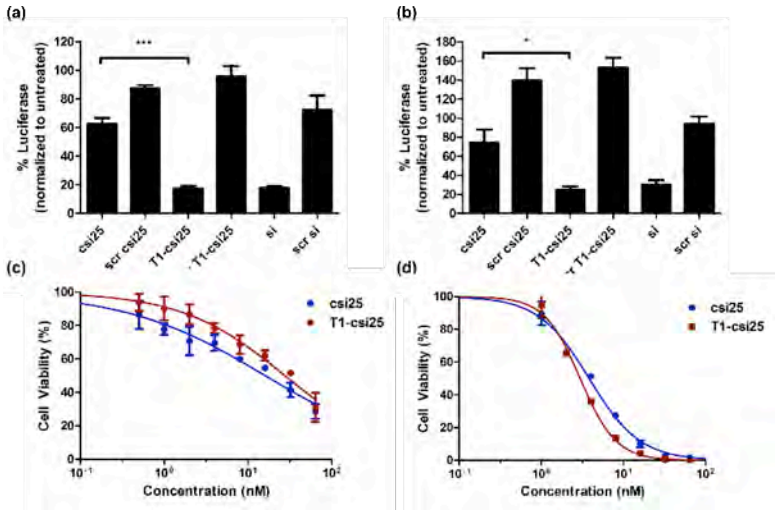

Figure 4. Luciferase knockdown by luciferase-targeting and scrambled (scr) csiRNA with a 25 bp stem (csi25), T1-csi25, and 21 bp siRNA with Mirus TransIT-X2 in luciferase-expressing (a) SKOV3 and (b) UCI101 cells, with normalization of luciferase expression to total protein. (c, d) Viability assays of csi25 and T1-csi25 with Mirus TransIT-X2 in SKOV3 cells (c) and UCI101 cells (d). Error bars represent standard errors of the mean of triplicates. *p<0.05, ***p<0.0001.

With the introduction of open ends into csiRNA, the resulting structure (T1-csiRNA) demonstrates greatly enhanced Dicer processing efficiency compared to csiRNA, with the majority of T1-csiRNA converted to 21-25 bp fragments after 24 hours of incubation with recombinant human Dicer (Figure 3d). Consistent with these results, T1-csiRNA demonstrated high silencing efficacy in SKOV3 and UCI101 cells *in vitro* (Figure 4a-b). With both Lipofectamine 2000 and Mirus Trans-IT X2, a polymeric transfection reagent, T1-csiRNA induced knockdown at levels significantly higher than for csiRNA and comparable to those for siRNA. Importantly, T1-csiRNA retained its cytotoxic effects at higher concentrations (Figure 4c-d), but gene knockdown can now be achieved at doses where minimal cytotoxicity occurs.

The advances in csiRNA design and our identification of its innate cytotoxicity will enable novel therapeutic strategies that target multiple ovarian cancer pathways with a single, programmable platform. We will focus next on optimizing a carrier for T1-csiRNA, which can potentially form more stable complexes than siRNA for systemic delivery. Our preliminary tests suggest that low molecular weight, biodegradable polymers give higher transfection efficiencies for csiRNA or T1-csiRNA than polymers that have higher molecular weight or are not degradable. We will therefore design and screen a library of biodegradable polymers, particularly poly(beta-amino esters), to determine the most suitable carrier for T1-csiRNA delivery. Further modifications of this optimized carrier will be studied to improve *in vivo* stability

of the T1-csiRNA complexes, including PEGylation of the polymer and modification of the complex with a polyanion coating. Following optimization *in vitro* in various ovarian cancer cell lines, we will test the *in vivo* performance of our T1-csiRNA system.

1.3 Development and testing of RNAi-MS targeting multiple genes.
The development of RNAi-MS incorporating more than one siRNA sequence was pursued to enable the delivery of RNAi targeting multiple oncogenic targets. To initially test this concept we assembled RNAi-MS containing siRNA sequences that target GFP and RFP. We initially confirmed that both sequences were incorporated into the same sponge by flow cytometry, and furthermore that the ratio of the two components could be precisely tuned by varying the reaction parameters. Treating the multi-RNAi-MS particles with different polycations, such as poly-L-lysine (PLL) and polyethylene imine (PEI), yielded nanoparticles that contained the multiple RNA sequences present in the orginial microsponge particles. The simultaneous knockdown two genes was assessed by transfecting HeLa cells expressing GFP and RFP with PEI-condensed particles. GFP and RFP expression both decreased with increasing doses of RNAi-MS while causing minimal toxicity to the cells. With 8nM of RNAi-MS we achieved 80% inhibition of RFP and 50% inhibition of GFP expression. The difference in the inhibition efficacy may be due to the increased stability and expression of GFP versus RFP. Initial experiments in luciferase-expressing ovarian cancer cells (SKOV3, CAOV3, and UCI101) have also shown promising gene silencing. With these initial results in hand, in year 3 we will further optimize this system for *in vivo* delivery and test the therapeutic efficacy of RNAi-MS targeting multiple nodes of the FGF pathway in an ovarian cancer mouse model in collaboration with the Birrer group at MGH.

Specific Aim 2: Modular LbL Nanoparticles for Temporal Release

Aim 2 Current Objective:
The objective of Aim 2 is to generate LbL nanoparticles containing chemotherapy drugs appropriate to OC in the core, with siRNA releasing layers formed around the nanoparticle core, and an external "stealth" layer that can facilitate systemic delivery and tumor targeting. The LbL nanoparticle approach is based on the concept of combination therapeutics in which siRNA is used to block specific survival pathways, followed by delivery of a chemotherapy drug that can then effect tumor cell death. The siRNA targets are specifically selected to take advantage of genetic pathways characteristic of advanced serous OC. We have selected chemotherapy drugs that induce DNA damage, and are of well-established use in OC.

Dr. Joyce Liu and Dr. Ronny Drapkin of the Dana-Farber Cancer Institute serve as the collaborators/unpaid consultants in this Aim to provide a clinical perspective and guidance in pre-clinical studies of specific combination drug/siRNA sets for targeting OC, and in the use of appropriate animal models to test these systems. The main focus of the Drapkin lab in the past few years has been deciphering the pathogenesis pathways of aggressive serous tumors; hence, his expertise is in the development/establishment of novel model systems that incorporate the fallopian tube (FT) as the true cell of origin for serous carcinomas.

Aim 2 Results, Progress, and Accomplishments with Discussion:

2.3 Combinations - Modulation of simultaneous and/or staged release of RNAi and chemotherapy drug from drug-loaded nanoparticles cores coated with siRNA LbL layers.

The design of "sheddable layers" on nanoparticle surfaces for synergistic combination drug therapy using modular chemical linkages to enable the tumor microenvironment pH or other

tumor cell-triggered sequential release of siRNA, followed by inhibitors and chemotherapy drugs from the nanoparticles. In Year 1, we addressed the ability to load LbL nanoparticles with different cargos, ranging from cisplatin to paclitaxel, with sufficient control for effective thereapies (Aim 2.1 – Modulation of Release of Chemotherapy Drugs).

Active targeting of nanoscale drug carriers can improve tumor-specific delivery; however, cellular heterogeneity both within and among tumor sites is a fundamental barrier to their success; in Year 1 we also addressed this challenge [9] (Aim 2.2 – Design and Tuning of Dynamic Stealth Layers) by developing an LbL nanoparticle architecture that targets solid tumors through three independent mechanisms: (i) size-dependent passive tumor targeting, (ii) active, ligand-directed targeting of cell-surface CD44 receptor, a well-characterized biomarker for cancer stem cells, and (iii) hypoxic tumor pH-responsive cellular delivery. Compared with nanoscale drug carriers of equivalent size and charge, these novel drug carriers enhanced tumor accumulation 4.0-fold while decreasing liver accumulation 2.0-fold. **In Year 2**, we examined whether these engineered polymer nanotechnologies could improve the safety and potency of rational combination therapies in which (i) cellular colocalization is required for efficient cell killing and (ii) dose-limiting toxic effects are compounded in combination.

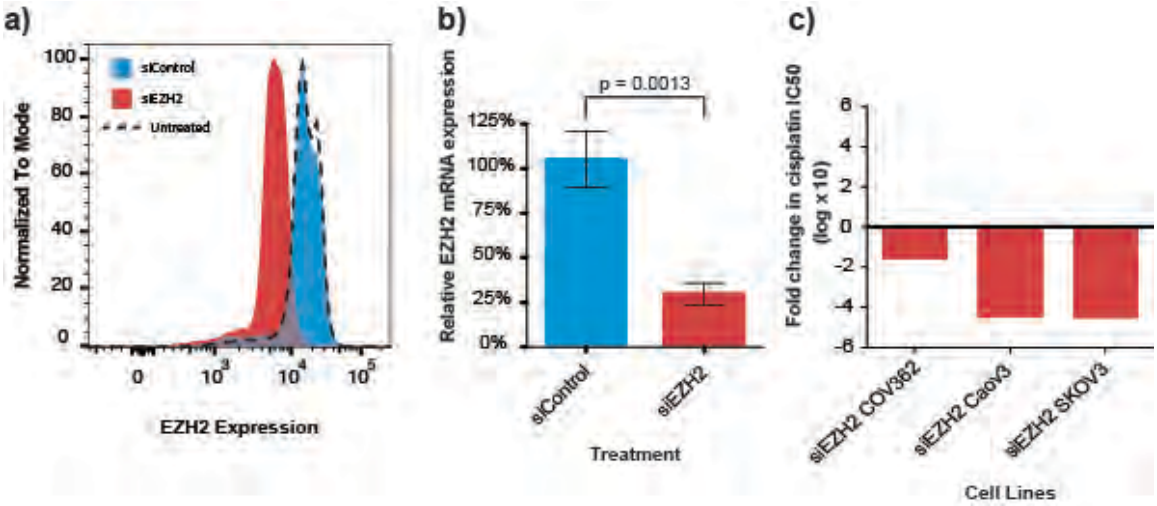

Figure 5. Depletion of EZH2 using siRNA sensitizes COV362, Caov3, and SKOV3 ovarian cancer cells to cisplatin. **a)** Caov3 cells were transfected with EZH2 siRNA (siEZH2) or control siRNA (siControl) and analyzed by flow cytometry 48 hours later to assess EZH2 protein levels. siEZH2 treated cells expressed less EZH2 relative to siControl treated cells and untreated cells. **b)** To further validate knockdown, RNA from siEZH2 and siControl treated Caov3 cells was analyzed by qRT-PCR. Significantly lower EZH2 transcript levels were observed in siEZH2 treated cells relative to siControl treated cells. Error bars represent SEM of three biological replicates. **c)** The fold change in cisplatin IC50 due to EZH2 depletion was determined for COV362, Caov3, and SKOV3 ovarian cancer cells using the Cell-Titer Glo viability assay.

Following consultation with Dr. Ronny Drapkin, we have chosen to pursue enhancer of zeste homolog 2 (EZH2) as a target for siRNA knockdown in serous ovarian cancer. EZH2 is overexpressed in OC and has been implicated in a host of oncogenic activities due to its role as an epigenetic modifier of gene expression. Beyond implications for enhancing metastasis and angiogenesis, EZH2 has also been linked to suppressing DDR-mediated apoptosis by instead promoting cell-cycle arrest through phosphorylation of Chk1. Entry into cell-cycle arrest may allow OC greater opportunity to repair damage caused by cisplatin. EZH2 further mutes the DDR by epigenetically down-regulating expression of Bim, a potent pro-apoptotic protein. Using

a chemically modified siRNA from Life Technologies, we have been able to potently downregulate expression of EZH2 at both the protein and transcriptional level (Figure 5a and b). Moreover, we have demonstrated the depletion of EZH2 increases sensitivity to cisplatin in OC cells (Figure 5c). This siRNA sequence is now being incorporated into a multi-drug LbL nanoparticle with OC targeting capabilities.

Screening of high grade serous ovarian cancer cells with PARP inhibitors, cytotoxic and anti-angiogenic agents for selective LbL nanoparticle combination therapy.

One of the main drawbacks of PARP inhibitor and cytotoxic drug combination therapy in clinical setting, is severe bone marrow suppression and systemic toxicity. PLL/HA functionalized liposome nanoparticles with ratiometric drug loaded combination therapy will selectively target CD44 in *BRCA1* mutant COV362. This in combination with a well-designed *in vivo* model system we believe will shed light on the ability of our system to circumvent the current clinical limitations in overcoming taxol-based drug resistance and inherent systemic toxicity associated PARP inhibitors and cytotoxic combination therapy. We hope to first test the potential of PARP inhibitor and cisplatin together in a single nanoparticle for BRCA1 mutant cancers. In future developments, we plan to incorporate siRNA in the outer regions of such nanoparticles that can silence genes so as to introduce BRCA1 mutant characteristics to OC cells, enabling the formulation to be effective to a larger set of sensitized cells.

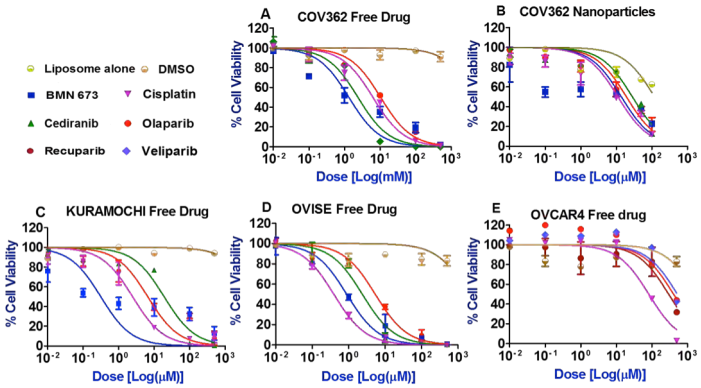

Figure 6. Screen in ovarian cancer cells. Treatment of high grade serous OC cells with PARP inhibitors, anti-angiogenic and cytotoxic agents to evaluate cell-type specific therapeutic efficacy. Cells were treated with increasing concentration of both free (**A,C,D,E**) and liposome PLL/HA drug encapsulated nanoparticles (**B**). Cells were treated for 96hrs and dose dependent IC50 values were calculated. Highest drug efficacy was observed in COV362 (*BRCA1* mutant) and KURAMOCHI (BRCA2 mutant) with BMN 673 in both cells lines. Data represents n≥3 independent experiments.

In Year 1 (2014), we developed the rationale for harnessing *BRCA1/2* mutation in serous ovarian cancer to elicit 'synthetic lethality' and also to overcome taxol-based drug resistance and relapse. We used western blot to screen a number of high grade serous ovarian cancer (OC) cells to identify their genetic profile and molecular biomarkers which is critical for cell-type specific designing of targeting and combination therapy. Four cell lines namely: COV362, OVCAR4 and OVCAR8 and negative control line OVISE were selected. More recently, we have also included KURAMOCHI, a high grade serous (*BRCA 2* mutant) cells, one of the best cell lines for modelling serous ovarian cancer, which we did not have last year in our screen. **In Year 2** (2015), we screened all the five cell lines against a number of PARP1/2 inhibitors namely: **Olaparib** (AZD2281), **Veliparib** (ABT-888), **Rucaparib** (AG014699), and **BMN 673** (talazoparib). We also tested **Cisplatin,** one of the taxol-based drugs of choice for OC therapy. In recent stage 2 and 3 clinical trials, the anti-angiogenic agent, **cediranib** (AZD2171) in combination with PARP inhibitor Olaparib has shown similar therapeutic benefit as observed in PARP inhibitor and Cisplatin combination therapy. We therefore included cedinarib in our cell line specific screen. We first screened all the above cell lines against five free drugs and calculated their respective IC50 values. COV362, KURAMOCHI and OVISE showed the lowest

dose with the highest efficacy (**Figure 6A,C D**), but not in OVCAR8 and OVCAR4 (**Figure 6E**). We therefore proceeded to teste liposome PLL/HA drug encapsulated nanoparticles in COV362 our top selected cell line. We observed IC50 values comparable with free drug (**Figure 6B**).

We observed strong DNA damage in cells treated with both free drug and in liposome nanoparticles (**Figure 7, B-F**), indicating that the encapsulation of drugs does not affect their potency. **Figure 7A**, shows cyro-TEM image of liposome alone nanoparticles (first panel), layered with PLL (middle panel) and with PLL-Avidin to mimic HA (last panel), to show the change in size and robust LbL formation. OV362 is therefore our cell line of choice for *in vitro* and *in vivo* drug combination and CD44 targeting therapy which is currently ongoing.

To effectively model high grade serous ovarian cancer which spreads as micro tumor models throughout the peritoneal cavity into the abdominal fat pads, omentum and the lymphatics drainage we have successful developed novel xenograft orthotropic model in female SCID/nude mice to mimic advanced serous OC disease pathology.

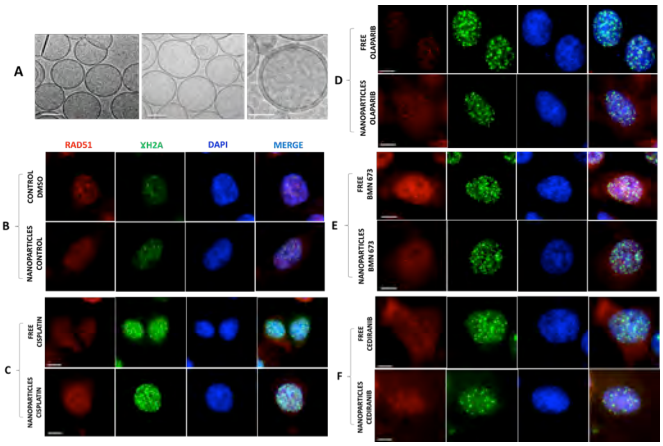

Figure 7: Formulation and evaluation of therapeutic efficacy of free and PLL/HA encapsulated nanoparticles (NP) drugs in inducing DNA damage in OC cells. Successful formulation of robust Liposome functionalized PLL/HA nanoparticles were fabricated for combination therapy and selective CD44 targeting in OC. **A.** plane liposome (first panel ~97nm), (middle panel, liposome with PLL ~110nm) and (last panel, liposome-PLL-Avidin ~110-120nm). Stable for 21days at 4^0C. COV362 cells were treated with free drug (all top panels) and drug encapsulated nanoparticles (NP) all bottom panels with **B**, controls; **C**, Cisplatin; **D**, Olaparib; **E**, BMN673, **F**, Cedinarib. Gamma H2A and RAD51 are makers for DNA foci in response to inhibition of HR and inducing of apoptosis upon drug treatment. Liposome PLL/HA formulated drug show similar efficacy as free drug.

Specific Aim 3: Ligand Cluster Syntehtic Polypeptide Nanocarriers: A New Approach to OC Molecular Targeting

Aim 3 Current Objective: Several studies of OC biomarkers suggest that there are specific cell surface receptors that are upregulated in ovarian tumors that can be used as molecular targets for therapeutic delivery. Results in our lab indicate for the first time that systems with optimized 3D clustering of ligand groups can lead to significantly higher intracellular uptake, as presented in a simple tumor model. Our early work used the folate receptor, which is overexpressed in many ovarian tumor cell lines; however, the systems can be tailored for individualized medicine approaches using different ovarian tumor surface biomarkers, including short peptides or antibody fragments selected to bind to specific receptors[10-12]. Higher order oligomer formation of epidermal growth factor receptor has been studied by many research groups [13]. Recent results from Liu, Livingston and coworkers have shown that ErbB3 receptor plays a significant role in tyrosine kinase signaling and tumor growth in OC primary tumors. The use of controlled multivalent interactions to bind these receptors in a manner that inhibits kinase signaling can lead to significant down-regulation of kinase signaling as well as enhanced uptake of the chemotherapy-loaded nanoparticles, thus providing a dual synergistic mechanism for tumor treatment that could significantly change the nature of targeted tumor treatments by blocking cell growth, inhibiting resistance and killing tumor cells with greatly enhanced efficacy and specificity. The objective of this work is thus to a) use a synthetic polypeptide based block

copolymer system to design nanoparticles with high efficacy in siRNA encapsulation and release, while b) furthering the design of the hydrated "exterior" block to enable the presentation of ligand-clusters on the surfaces of the resulting assembled polymer nanocarriers via the use of linear-linear and linear-dendritic block copolymers. The approach will include the design of dendritic macromolecules that are functionalized with the EGF protein or a corresponding short peptide that binds EGF and/or the Neuregulin 1b peptide domain. We will examine components of these block copolymer systems as modular elements that can be combined for optimal targeting of OC tumors and siRNA efficacy. Unpaid collaborators and consultants in this project include Michael Yaffe of the Koch Institute, MIT.

3.1 Synthesis and characterization of new synthetic polypeptide conjugates: Neuregulin ligand clustered linear-dentritic polypeptide block copolymer to target ErbB3 in high grade serous ovarian cancer

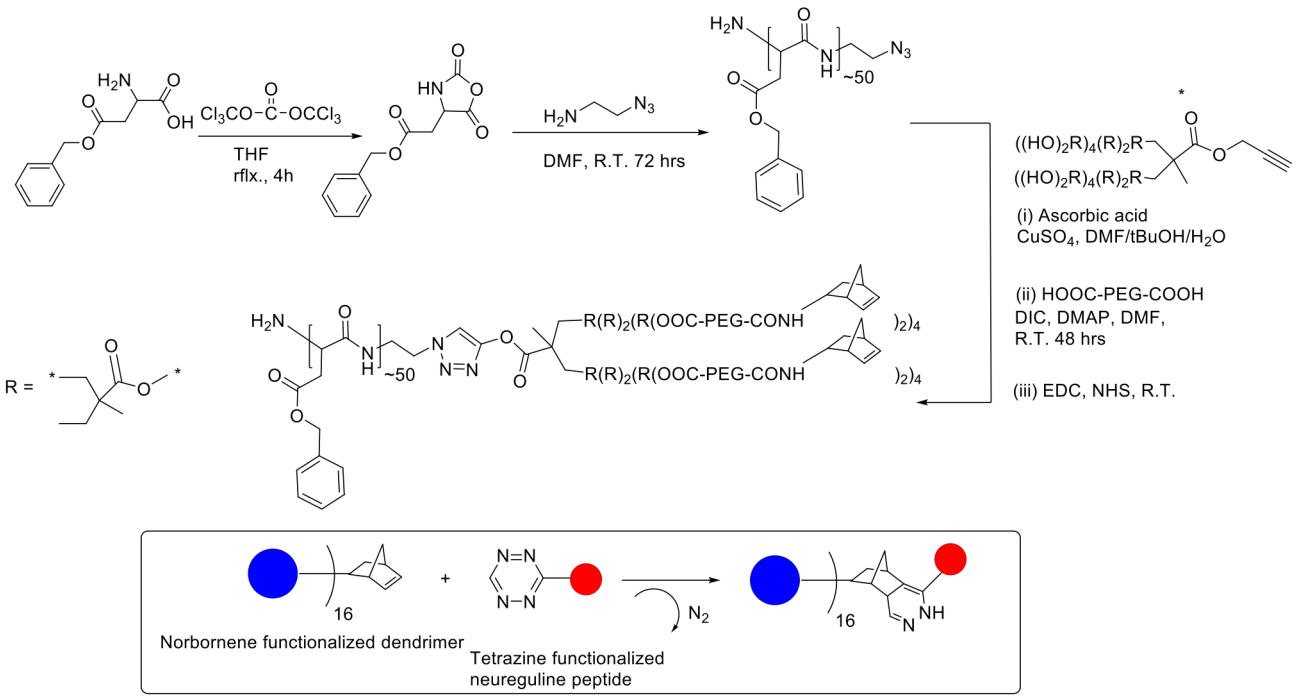

Figure 8. Synthesis of peptide amphiphiles for RNA interference-mediated chemosensitization of HGSOC. Neuregulin congugation: 6 each.

The autophosphorylation of growth factor receptors and activation of tyrosine kinase can lead to signaling pathways for cell growth, motility and morphogenesis. ErbB3 plays a significant role in the autocrine loop that may have large significance particularly for serous ovarian tumor growth. Neuregulin (NRG) is peptide ligand bind to extracellular domain of ErbB3. Bivalent NRG ligand with an amino-acid linker spacer exhibit potent antagonism that suppress the ErbB3-NRG dimerization and reduce the downstream signaling pathways of ErbB3. However, NRG in free monomer form does not block ErbB3; instead, it results in phosphorylation of ErB3, leading to undesired cell proliferation. **In Year 2**, we have synthesized multivalent (conjugation # = 6) neuregulin (NRG) functionalized linear-dentritic polypeptide copolymer to investigate the ligand clustering effect on NRG binding to ErbB3 receptors (**Figure 8**). The phosphorylation of ErbB3, AKT, and ERK signaling pathways are reduced by the NRG ligand clustered nanoparticles whereas monovalent NRG gave strong stimulation (**Figure 9**) in a high ErbB3 expression breast cancer cell line. We have demonstrated that NRG clustered nanoparticles target ErbB3 to reduce the signaling pathway responsible for cancer cell survival and growth.

Current efforts include further downstream signaling suppression tests using other high-grade serous ovarian cancer cells with ErbB3 over-expression. Furthermore, we will test the enhanced intracellular uptake behavior and downstream signaling suppressing ability using the chemotherapeutic loaded nanoparticles. Therefore a dual synergistic mechanism for tumor treatment can be achieved by blocking signaling pathways that are responsible for cell growth, thus killing tumor cells with greatly enhanced efficacy and specificity due to the 3D clustering ligand presentation. Lastly, proof-of-principle work will be conducted in pre-clinical animal models of high-grade serous ovarian tumors.

A

conc. (nM)	pErB3	pAKT	pERK
0	1	1	1
0.17	0.77	0.88	0.8
0.5	0.71	0.90	0.7
1.5	0.75	0.99	0.8
4.5	0.48	0.90	0.4

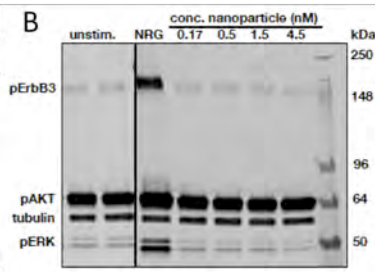

Figure 9. A) quantification of downstream signaling pathways (pErbB3, pAKT, and pERK) reduced by NRG clustered peptide amphiphile nanoparticles at various concentrations (0, 0.17, 0.5, 1.5, 4.5 nM), B) westen blotting of downstream signaling pathways after NRG clustered nanoparticles treatment using NRG monovalent ligand and unstimulated basal condition as controls.

3.2 Engineered peptide amphiphiles chemosensitize metastatic tumors to frontline chemotherapy via RNA interference.

In Year 1, we sought to exploit tumor suppressor deficiency as a means to treat HGSOC patients with metastatic tumors. We identified the kinase, MK2, as a key regulator of DNA damage response – selective to p53 deficient tumor cells (96% of HGSOCs[14]) – and showed for the first time that p38/MK2 signaling is required for survival in ovarian cancer cells treated with platinum (frontline chemotherapy for HGSOC patients). Because MK2 is currently undruggable and also required for tolerance to systemic chemotherapy, we engineered and series of amphiphilic peptides that self-assemble with oligonucleotides into nanoscale macromolecular structures that target the delivery of siRNA to HGSOC tumors *in vivo*. Engineered polypeptides were synthesized as via *N*-carboxyanhydride (NCA) mediated ring-opening polymerization of side-chain protected amino acids (**Figure 10**). siRNA complexes targeted ca.5% of total tumor burden, as well as tumor stromal cells *in vivo*. **<u>In Year 2</u>**, we assessed the capacity of these constructs (**Figure 10a**) to treat HGSOC *in vitro* and vivo. We developed mouse models of FIGO Grade III[15] HGSOC (**Figure 10b**), with intraperitoneally disseminated disease, and Grade IV, with distant lung metastases. Engineered peptide amphiphiles increased the serum stability of siRNA two-fold and facilitated *in vitro* mRNA silencing equivalent to and better than commercial transfection reagents (**Figure 10c,d**). *In vivo*, peptide amphiphile nanoparticles-mediated silencing of MK2 in lung metastases and induced growth arrest in intraperitoneal metastases (**Figure 10e-g**).

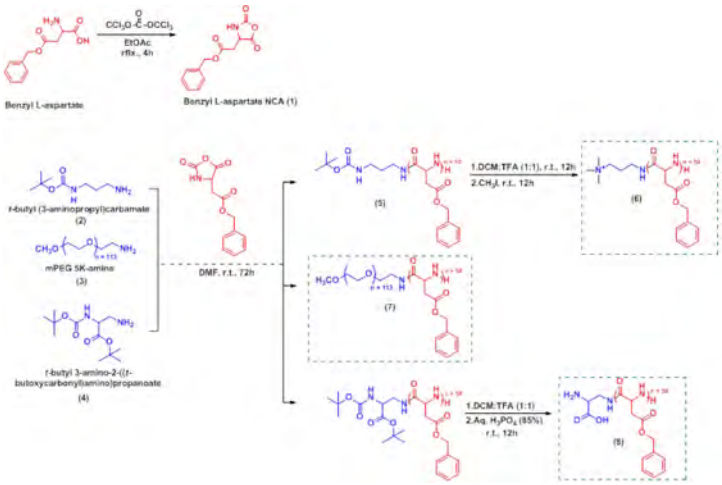

Figure 10. Synthesis of peptide amphiphiles for RNAi-mediated chemo-sensitization of HGSOC. The highly reactive monomer Benzyl-L-aspartate NCA is polymerized by bis-functionalized nucleophilic macroinitiators. Deprotection and post-functionalization of the resulting polymer yielded synthetic polypeptides with cationic, neutral and zwitterionic terminals.

Further, we found that peptide-mediated MK2 RNA interference chemosensitized metastatic tumors to platinum *in vivo* (**Fig 3.4h,i**) and that concurrent silencing of the platinum-adduct translesion polymerase, REV3, elicited growth arrest in mice bearing metastatic lung tumors. Current investigations focus on the characterization of cytokine activation following repeat dosing, serum biochemical markers of effects on vital organs, histopathological examination of vital organs and tumor tissues, immunohistochemistry of tumor/stroma, and longer-term efficacy in intraperitoneal mouse models of Grade III HGSOC.

3.3 The presentation of ligand on nanoparticle surfaces in cluster arrangements that enable optimization of ligand presentation at low overall ligand levels and maximized tumor cell selectivity.

In Year 2, we have standardized a facile synthetic protocol to construct nanoparticles with hydrated exterior via the use of linear-linear and linear-dendritic macromolecules. In a spatially controlled, clustered pattern, the exteriors of these nanoparticles were functionalized with short peptide (GE 11, NH_2-Tyr-His-Trp-Tyr-Gly-Tyr-Thr-Pro-Gln-Asn-Val-Ile-COOH**)** that binds to EGF. We have previously showed that, clustered arrangement of ligands on nanoparticle surface can substantially increase interactions between cell and substrate (nanoparticles in our case), and not only the valency, but also spatial factors such as branching mode and density of ligands per cluster are important in binding to target cells, and downstream signal processing (Angew. Chem. Int. Ed. 2010, 49, 7266-7270). We have taken the advantage of dendritic macromolecules to engineer systematic and informed linear-dendritic block copolymer systems with distinct hydrophobic and hydrophilic domain and used mixed micceller technology to generate nanoparticles with clustered arrangement of surface ligands. An idealistic representation of such construct is shown in **Figure 11a**. Hydrophobic PBLA (poly-benzyl L-aspartate) comprised the linear block, to which a

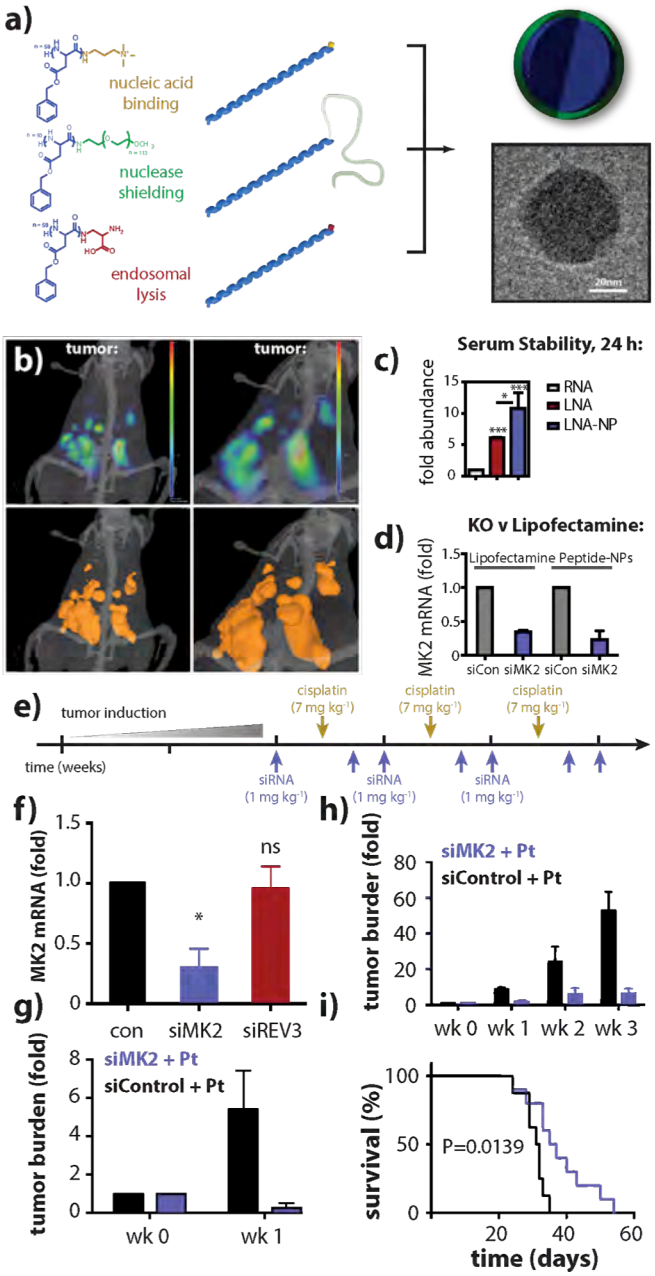

Figure 10. Engineered peptide nanotechnologies chemosensitize tumors to platinum therapy. a) In the presence of small interfering RNA, engineered peptide amphiphiles self-assemble in sub-100 nm nanoscale particles as shown by cryogenic transmission electron micrsocopy. b) Bioluminescence tomography of high-grade serous ovarian cancer (HGSOC) tumor models. c) Enhanced serum stability from nanoparticle-complexed siRNA and (d) *in vitro* mRNA silencing equivalent to commercial transfection reagents. e) RNAi combination chemotherapy treatment regimen and (f) *in vivo* mRNA silencing in orthotopic tumor transplants. g) RNA interference combination chemotherapy induces growth arrest in HGSOC tumor models. h) Treatment response and (i) improved survival in orthotopic tumor transplant models.

generation four, hydrolytically degradable PEGylated polyester dendron was attached. We have established a copper-free click chemistry protocol to generate this architecture, by which the linear segment can be easily and reproducibly attached to the dendritic block. Using carbodiimide/N-hydroxysuccinimide (EDC/NHS) chemistry, we have attached well-characterized EGF ligand, GE 11 on the dendritic termini at different percentage of functionalization, and confirmed the success of conjugation by UV-Vis spectroscopy. Stoichiometric mixing of GE 11 functionalized block copolymers with their non-functionalized analogue resulted in the formation of GE 11 decorated micelles (**Figure 11b**), where the ligands are distributed in a spatially clustered pattern on the nanoparticles surface. As a proof-of-concept, we evaluated the efficacy of these architectures to target EGFR expressing cells, such as MDA-MB-468. Both flow cytometric and confocal microscopic investigation revealed that, nanoparticles with cluster arrangement of 40/30, i.e. 40% GE 11 functionalized polymers constituting 30% of the micelle formulation (with optimal size of ca. 8 GE 11 per cluster) showed significantly enhanced uptake in EGFR expressing cell lines. Hence, in the past year, the mechanism and synthetic route to immobilize ligands on nanoparticle surfaces in clustered arrangements have been established, which has enabled optimization of ligand presentation at low overall ligand levels. Ongoing work is focused on deconvoluting the mechanism of such enhanced uptake, and translating these regio-controlled, ligand-decorated linear-dendritic nanoparticles as drug carriers for in vivo applications in ovarian cancer setting for maximized tum

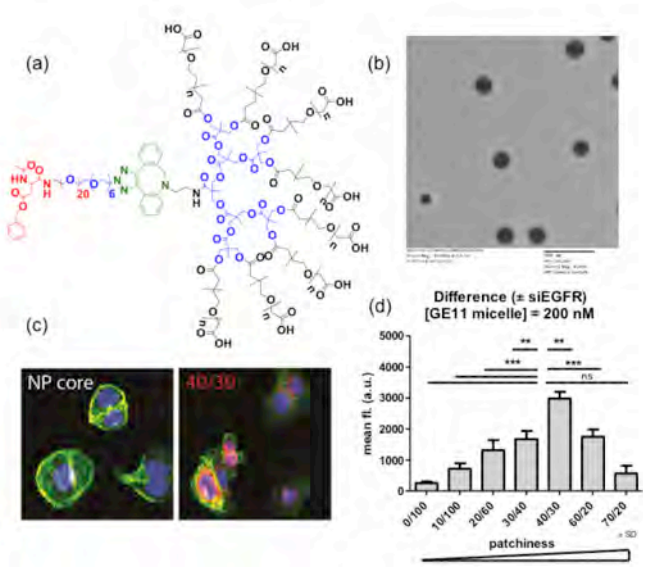

Figure 11. Engineered linear-dendritic block copolymer for optimized ligand presentation. a) Representative structure of a linear-dendritic block copolymer where the linear block is attached to the dendritic segment through DBCO-mediated copper-free click chemistry. PEGylation of dendritic block generates the caboxylic acid handles for ligand functionalization through EDC/NHS chemistry. Generation 4, i.e. 16 endgroups containing block copolymer has been used for the actual study b) Stoichiometric mixing of functionalized and unfunctionalized amphiphlies generates self-assembly driven miceller nanoparticle of ~100 nm diameter, Scale bar = 500 nm c) Confocal microscopy image of MDA-MB-468 cells revealing micelles prepared from 40% GE 11 functionlized nanoparticle constituting 30% of the formulation (40/30) exhibiting enhanced cellular uptake (d) Flow cytometric investigation demonstrated that, of different nanoparticle formulations, those with the presentation of 8 GE11/cluster (40/30) showed enhanced cellular uptake as observed by a difference between the cell-associated fluorescence in EGFR expressed and knocked-down MDA-MB-468 cell lines.

OCRP Ambassador: As the Teal Innovator Awardee, I have chosen to engage with the National Ovarian Cancer Coalition (NOCC) through its national and local chapter. By using the MIT Koch Institute as a platform, and working with NOCC and other local organizations, I organized an Ovarian Cancer Symposium, "Bridging the Gap in Ovarian Cancer" (http://ki.mit.edu/news/events/ocsept2014) for survivors and advocates and the general public through the Koch Institute at MIT that addressed and showcased the role of new technologies in addressing ovarian cancer, as well as provide a panel of experts to discuss the challenges and new developments designed to help treat disease. This symposium took place with great success on September 16, 2014. The workshop was used to inform the public about the importance of funding research for ovarian cancer, and contribute to raising support and the profile of ovarian cancer. Several ovarian cancer survivors and advocates attended, as well as

research scientists and the ovarian cancer research community. The talks focused on new research developments in ovarian cancer, described in a manner that was accessible to a general audience, followed by a panel of ovarian cancer survivors, clinicians and researchers. The videos of this event are available online (http://techtv.mit.edu/collections/kochvideos:3777) to the public.

KEY RESEARCH ACCOMPLISHMENTS:

- A greater understanding of the csiRNA has led us to discover that it can be used as a means of achieving additional lethality to ovarian cancer cells through the activation of the tumor immune response. Additionally, we have shown that this response can be designed to be highly specific to cancer cells when delivered using a polymeric delivery vector.
- We have found that by treating csiRNA with specific enzymes, it is possible to modify the macromolecules so that they are much more readily broken down in cells to derive siRNA hairpin molecules that yield a high degree of knockdown, a development key to generating more effective synergistic therapies. This finding, in combination with the impact of immune activation, will be examined to achieve highly synergistic and selective means of ovarian cancer cell death.
- The generation of LbL nanoparticles containing combinations of drugs, inhibitors and siRNA have been established in our laboratory. We have shown early indications of efficacy against a range of representative ovarian cancer cells using LbL nanoparticle systems with cisplatin or with cisplatin and additional inhibitors. Further work in this area is anticipated to lead to new combination drug nanotherapies.
- Synthetic polypeptide block copolymers have been designed to very effectively encapsulate both siRNA and a small molecule drug inhibitor, while yielding high siRNA knockdown efficiency. We have developed an orthotopic ovarian cancer tumor model in which these nanoparticles, in combination with cisplatin, show very promising efficacy against high grade ovarian cancer. These animal studies are now in their last stages. Development of this dual treatment for ovarian cancer targets MAPK2 and the DNA damage response pathway through siRNA against MK2 and cisplatin.
- We have demonstrated that NRG clustered nanoparticles target ErbB3 to reduce the signaling pathway responsible for cancer cell survival and growth.

CONCLUSIONS

At the close of the second year of funding, we have made significant progress in the development of each of our three key siRNA delivery platforms that offer unique advantages in addressing ovarian cancer.

New developments with csiRNA and continued work with the related RNAi microsponges is promising, and greater understanding of these systems will enable the investigation of targets identified by Michael Birrer, including FGF18, that will synergize with the innate immune response of the tumor cells to achieve a highly effective treatment.

We continue to develop LbL nanoparticle systems designed specifically to deliver a chemotherapy drug for DNA damage from its interior, and siRNA that can block genes that promote or enable cell survival in the presence of the drug from its exterior. New work in this area includes the successful knockdown of EZH2 and other select targets as a result of collaborations with the Drapkin lab. In vitro work suggests potential for efficacy in these systems, and we will examine the efficacy in vivo in the upcoming year, in particular when using LbL stealth targeting outer layers also developed during this grant period.

We have developed a cluster-ligand system that seems to lead to downregulation of pathways associated with tumor cell growth and proliferation, which is key to the objective of

Aim 3. Our synthetic polypeptide linear and linear-dendritic block copolymer have been demonstrated in vivo to yield promising results in a high grade serous ovarian cancer model, and these systems will be further examined to confirm proposed mechanisms of action.

PUBLICATIONS, ABSTRACTS AND PRESENTATIONS:

Journal Articles:

Dreaden, E.C.; Kong, Y.W.; Morton, S.W.; Echevarria, S.C.; Drapkin, R.; Yaffe; M.B.; Hammond, P.T., An Engineered Layer-by-Layer (LbL) Nanoparticle for Tumor-Targeted Synergistic Blockade of MAPK and PI3K, **2015**, *in press, Clinical Cancer Research.*

Roh, Y.H.; Lee, J.B.; Shopsowitz, K.E.; Dreaden, E.C.; Morton, S.W.; Poon, Z.; Hong, J.; Yamin, I.; Bonner, D.K.; Hammond, P.T., Layer-by-Layer Assembled Anti-Sense DNA Microsponge Particles for Efficient Delivery of Cancer Therapeutics. *ACS Nano*, **2014**, 8(10), 9767-9780.

Shah, N.J.; Hsu, B.B.; Dreaden, E.C.; Hammond, P.T., Engineering Layer-by-Layer Thin Films for Multiscale and Multidrug Delivery Applications. In *Layer-by-Layer Films for Biomedical Applications*; 1st Ed. Picart, C.; Caruso, F., Voegel, J.-C., Eds.; Wiley-VCH: Weinheim, **2014**. ISBN: 978-3-527-33589-3.

Dreaden, E.C., Morton, S.W.; Shopsowitz, K.E., Choi, J.H.; Deng, Z.J.; Cho, N.-J.; Hammond, P.T., Bimodal Tumor-Targeting From Microenvironment Responsive Hyaluronan Layer-by-Layer (LbL) Nanoparticles, *ACS Nano*, **2014**, *8***,** 8374-8382.

Shopsowitz, K.E.; Roh, Y.H.; Deng, Z.J.; Morton, S.W., Hammond, P.T., RNAi Microsponges Form through Self-Assembly of the Organic and Inorganic Products of Transcription. *Small* **2014**, *10*, 1623-1633.

Roh, Y. H., Lee, J. B., Shopsowitz, K. E., Dreaden, E. C., Morton, S. W., Poon, Z., Hong, J., Yamin, I., Bonner, D. K., Hammond, P. T. Layer-by-Layer Assembled Antisense DNA Microsponge Particles for Efficient Delivery of Cancer Therapeutics. *ACS Nano*, *In revision* (nn-2014-02596b.R1)

In preparation:
Lawrence B. Mensah., Mohi Quadir, Aysen Richison, and Paula T. Hammond. Adapting fourth and fifth generation polyurea dendrimer as a novel drug delivery carrier for ovarian cancer therapy, 2015 *(manuscript in preparation).*

Presentations:
Mensah, Lawrence B.; Mohi Quadir.; Stephen Morton.; Jason Deng.; and Paula T. Hammond. Using Layer-by-Layer nanoparticles for novel ovarian cancer therapy. MIT, Koch Institute Fall Retreat, Cape Cod, MA, 2014. (poster)

Dreaden, E.C.; Kong, Y.W.; Yaffe, M.B.; Hammond, P.T.; Chemosensitizing Metastatic Tumors with Peptide Amphiphile-Mediated Silencing of p38/MK2 Pathway Signaling. **Gordon Research Conference** on Cancer Nanotechnology. 2015 July 28 – June 3, West Dover, VT.

Dreaden, E.C.; Nanoscale Biomaterials for Rational Combination Therapies against Metastatic Solid Tumors. Invited Seminar, **Ecole Polytechnique Fédérale de Lausanne**, Institute of Materials. 2015 Mar 17, Lausanne, Switzerland.

Dreaden, E.C.; Kong, Y.W.; Yaffe, M.B.; Hammond, P.T.; Self-Assembled Polymer Nanomedicines for Synergistic and Synthetic Lethal Drugging of Breast and Ovarian Tumors. Annual Meeting of the **American Institute of Chemical Engineers**. 2014 Nov 16-21, Atlanta, GA.

Dreaden, E.C.; Kong, Y.W.; Yaffe, M.B.; Hammond, P.T.; Drugging Metastatic and Locally-Disseminated Solid Tumors Using RNAi Combination Chemotherapy. Annual Meeting of the **Biomedical Engineering Society**. 2014 Oct 22-25, San Antonio, TX.

Dreaden, E.C.; Morton, S.W.; Deng, J.; Hammond, P.T.; Layer-by-Layer Nanoparticles: Rational Delivery of Rational Drug Combinations. Fall Meeting of the Materials Research Society. 2013, Dec 1-6, Boston, MA.

Dreaden, E.C.; Morton, S.W.; Deng, J.; Hammond, P.T.; LbL Nanoparticles for Combination Cancer Therapies: Receptor Targeting and Microenvironment Response. Annual Meeting of Biomedical Engineering Society. 2013, Sept 25-28, Seattle, WA. (podium talk)

Dreaden, E.C.; Morton, S.W.; Deng, J.; Shopsowitz, K.E., Hammond, P.T.; Layer-by-Layer (LbL) Nanoparticles for Active Targeting of Tumor-Initiating and Drug-Resistant Breast Carcinoma. 2013 Dana-Farber Cancer Biology Departmental Retreat. 2013, July 26, Boston, MA.

Dreaden, E.C.; Morton, S.W.; Deng, J.; Shopsowitz, K.E., Hammond, P.T.; Active Targeting of Triple-Negative Breast Tumors Using Hypoxia-Responsive Layer-by-Layer Nanoparticles. Gordon Research Conference on Cancer Nanotechnology. 2013, July 14-19, West Dover, VT.

Shopsowitz, K.E.; Morton, S.W.; Dreaden, E.C; Hammond, P.T; Self-Assembled Nanoparticles of Enzymatically Generated Polymeric siRNA. Annual Meeting of Biomedical Engineering Society. 2013, Sept 25-28, Seattle, WA. (poster)

INVENTIONS, PATENTS AND LICENSES:

Dreaden, E.C.; Hammond, P.T.; 2014 April. Nanotechnologies for Tumor-Targeted Horizontal Blockade of MAPK and PI3K. Pending.

Dreaden, E.C.; Hammond, P.T.; 2014 April. Multimodal Tumor-Targeting Polyelectrolyte Drug Carriers. Pending.

REPORTABLE OUTCOMES:

- Use of csiRNA to activate the immune response specifically in ovarian cancer cells to lead to heightened cell death.
- Multi-RNAi microsponge systems capable of transfecting two or more RNAi sequences with high efficiency using csiRNA, which can be adapted to synergistic ovarian cancer targets.

- Linear-dendritic block copolymers designed to present ligand in a manner that downregulates the EGF receptor while targeting drug nanoparticles.
- New linear-peptide block copolymers for delivery of siRNA/inhibitor combinations that show unique efficacy in HGSOC in combination with platinum drugs.

OTHER ACHIEVEMENTS:

- P.T. Hammond, appointed Department Head, Dept. of Chemical Engineering
- P.T. Hammond, recipient, Alpha Chi Sigma Award, American Institute of Chemical Engineering (AIChE), received November 2014
- E.C. Dreaden, Chair, ACS 2015 Symposia: 'Biological and Biomedical Polymers'
- E.C. Dreaden, High School Research Mentor, MIT, P. Gallagher
- P.T. Hammond, Elected Fellow of American Academy of Arts and Sciences, October 2013
- P.T. Hammond, recipient, AIChE Stine Award in Materials Science, November 2013
- P.T. Hammond, Vice Chair, Gordon Conference on Drug Carriers, August 2014
- E.C. Dreaden, NIH Ruth L Kirschstein Postdoctoral Fellowship (F32), 2013-2015
- E.C. Dreaden, Koch Institute Image Award, MIT, 2014-2015
- E.C. Dreaden, Co-Chair, BMES 2013 Symposia: 'Nanotechnologies for Cancer Detection and Treatment' 2013
- M.A. Quadir, Recipient of the S. Leslie Misrock Frontier Research Fund for Cancer Nanotechnology, 2015 (Postdoctoral Fellowship).

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